THE HINE LABORATORIES
RESEARCH AND DEVELOPMENT
1099 FOLSOM STREET · SAN FRANCISCO 3, CALIFORNIA

TOXICOLOGIC EVALUATIONS OF HEXANITROETHANE

FINAL REPORT

Conducted at the request of

The Lockheed Aircraft Corporation

Missile and Space Division

Sunnyvale, California

THE HINE LABORATORIES
RESEARCH AND DEVELOPMENT
1099 FOLSOM STREET · SAN FRANCISCO 3, CALIFORNIA

TOXICOLOGIC EVALUATIONS OF HEXANITROETHANE

FINAL REPORT

Conducted at the request of

The Lockheed Aircraft Corporation,

Missile and Space Division

Sunnyvale, California

Cleared for Item 6/6/63 by R. G. Gilman

Cleared 11/12/64 Rec'd per Gilman

1 December, 1961

PERSONNEL WORKING ON THIS PROJECT

William Blucher

L. Campbell, Ph.D.,

Donald Decker

M. L. Dewey, B.S.

Gaye Eisenlord, B. A.

C. H. Hine, M.D., Ph.D.

G. S. Loquvam, M.D.

D. Ludlow

Approved: C H Hine

C. H. Hine, M.D., Ph.D.
Director,
The Hine Laboratories, Inc.

TABLE OF CONTENTS

Section Number | Page Number

7.0 TABLES (Continued)

1.0 ABSTRACT

Further evaluation of the toxicity of Hexanitroethane has been carried out through a series of studies including repeated vapor exposures to three air levels of four species of animals, short term exposures to saturated vapors, repeated application to the skin of rabbits and sensory responses of human volunteers. Evidence of toxicity as reflected by mortality and decreased weight gain was present in all species exposed repeatedly at levels of 3.0 ppm in the ambient air.

Some evidence of physiological stress appeared in all species exposed to 1.0 ppm, as indicated by one or more of the following: mortality; increased weight of the lung; pulmonary, liver and kidney pathology; changes in the total blood urea nitrogen. No significant effects were produced on the circulating blood or on its production. Pathologic changes were primarily limited to the respiratory tract, in which a number of structural alterations were observed. Liver and kidney damage was seen inconstantly, and generally only at high levels. The 0.3 ppm level was essentially free of untoward effects. Exposures to the saturated vapors of the compound was markedly deleterious for periods of more than 20 minutes in rats. No significant irritation was produced by repeated skin contact with the materials.

Sensory threshold studies were conducted which indicated that eye irritation was a significant warning sign of undesirable concentrations. A suggested maximal allowable concentration in the air based on the above studies would be 0.1 ppm. In the absence of adequate ventilation to achieve this level, protective respiratory devices would be required for safe handling.

2.0 INTRODUCTION

This study was carried out at the request of Dr. Howard Kindsvater and Mr. H. J. Stone of the Lockheed Corporation, Missile and Space Division, Sunnyvale, California, under work purchase order No. 56-09365. The contract was arranged through the Radiation Detection Company of Palo Alto, California.

The purpose of the study was to explore certain aspects of the toxicity of the candidate rocketfuel Hexanitroethane (HNE) in order to evaluate the possible health hazards for personnel working with the compound, and to establish a basis for predicting safe air concentrations. Reference is made to our initial report on acute toxicity of HNE, submitted to Lockheed Missile and Space Division on October 15, 1960. The current investigation was directed along four major lines.

The first involved an assay of the potential toxicity from repeated inhalation of the vapors of HNE by the means of a 90-day exposure of rats and a subacute exposure of 25 days in three other species; rabbits, mice, and guinea pigs.

Attempts to arrive at the probable mechanism of toxic action of the material were made by observation of the physiological effects of the material on the intact animal, and by an evaluation of the morphological changes found on autopsy of severely intoxicated animals. The potential untoward effects from repeated skin contact was determined following repeated application of the material to the skin of rabbits through observations of the skin changes. Assessment of the warning properties of the material was made by exposure of a group of volun-

teers to vapor concentrations of the material and determination of their sensory responses. The experiments were carried out at the Hine Laboratories, Inc., in San Francisco, from the period 1 March through 15 November, 1961.

3.0 METHODS

The general methods applicable to handling of the different species are described in this section. Animals were housed in the common animal area measuring 60' x 25'. Fresh air to this area was provided on a continuous basis, being filtered through a MSA filter capable of removing particles down to 0.1 micron in size. The temperature in the area was controlled by thermostat which allowed heating, but not cooling of the air. Forced exhaust of the area was obtained through a separate ventilating system. The temperature was maintained at 72° F. minimum, and did not exceed 80° F. in the housing area.

Animals were kept in this area at all times except during exposure periods, at which times they were transferred to the exposure area on the third floor of the laboratory. Exposures took place in a steel cylindrical chamber, of approximately 200 liters capacity. Air was drawn through the chambers by negative suction, and metered through standard rotometers. The air exchange varied between 4 and 12 changes per hour. The HNE vapors were volitalized by passing air over solid HNE powder which was mixed with glass beads to form a maximum surface area in a glass tube 15 x 200 mm.

The volume of air passing through the HNE tube, and that passing to the chamber were balanced empirically to give the desired concentration. Laboratory air was filtered through glass wool prior to passage over the HNE, but was not otherwise treated; no filtration of the main diluting stream was made. No other chemical materials were present in the exposure room other than those used for sampling the HNE vapors. The temperature of the exposure room varied between 65° F. and 90° F with a mean temperature of 76° F.

Daily checks were made on HNE concentrations within the chamber and the relative air flows adjusted as required. Since the quantity of HNE vapor depended in part on the ambient temperature, measurements were made more frequently on warm days.

During their time in the exposure chambers, the rats, mice, and guinea pigs were housed in separate compartments of large, drawer-type, steel trays with multiple perforations. The rabbits were allowed free movement within the chamber. Rats were exposed separately; rabbits, mice, and guinea pigs were exposed together.

Analysis of HNE vapors was carried out according to the technique developed in this laboratory.

Rabbits used in the skin irritation study were housed in the same area. At the time during which the material was placed on the skin, they were confined in individual holders. These were placed in a hood in the exposure room.

3.1 METHODS FOR DETERMINATION OF VAPOR CONCENTRATION OF HEXANITROETHANE

Reagents:

1. Standard solution of HNE: Accurately weigh 500 milligrams of HNE into a 100 ml. volumetric flask and make to volume with n-Hexane. Batch L-II was used as primary standard.

2. Working standard: 10.0 ml. of standard HNE is diluted to 100 ml. with n-Hexane . 1 ml. = 500 mcg HNE.

3. Color development reagent: Dissolve 250 milligrams of acenaphthene in 250 ml. of benzyl alcohol. Add 5 drops of a saturated solution of sodium hydroxide and shake vigorously.

Preparation of Standard Curve.

Prepare a series of tubes containing 100, 200, 300, 400, and 500 micrograms of HNE standard. Add 10.0 ml. of color developing reagent and shake. Determine percent transmission using a Bausch and Lamb Spectronic 20 at a wave length of 470 mμ against a reagent blank. The standard curve is linear throughout the range of 100 to 800 micro-- grams of HNE. The color is stable for 24 hours. Turbidity will develo after several hours if the reagent is exposed to atmospheric humidity.

Determinations of Air Concentration:

In collecting air samples, all glass absorbers were used. These consist of a 50 ml. glass stoppered container with standard taper groun glass connections at the entrance and exit tubes. A sintered glass tip of coarse porosity is fused to the end of the gas entrance tube at the bottom of the inlet tube.

10.0 ml. aliquots of the color developing reagent were used as absorbing solutions. The flow of air containing HNE from the exposure chambers was metered, using a precision wet test meter. Samples were drawn as it bubbled slowly through the absorbing solutions, using negative pressure from a suction pump. Five, ten, and fifteen liter air samples were taken respectively from the three chambers, theoretically containing 3.0, 1.0, and 0.3 ppm. HNE. Fifteen liter samples from the control chamber were also analyzed periodically for interferences.

The entire sampling arrangement consisted of exposure chamber, glass absorber containing developing reagent, wet meter, and vacuum pump connected in series. This arrangement permitted frequent sampling of the exposure chambers without interruption of the exposure period.

At the termination of each metered sampling period, the color reagent was transferred to Spectronic 20 tubes and percent transmissions determined at 470 mμ, using a reagent blank to set the instrument. Concentrations were calculated using a standard curve prepared from Batch L-II of HNE.

3.2 DETERMINATION OF VARIOUS LOTS OF HNE

Three lots of HNE were received during the course of the study; Batch L-II, Batch L-VIII, and one lot having no batch number.

Lot L-II was used as the primary standard in the preparation of the standard curve. The other two lots were compared to it using the same procedure as that employed for the standard curve.

3.3 REPEATED VAPOR EXPOSURES TO RATS

60 male and 60 female Long-Evans rats obtained from the Animal Supply Company of Napa, California, were separated according to sex and housed in the general animal area for a period of two weeks prior to introduction into the experiment. They were then randomized into four groups of 15 each, and housed 3-4 to a cage. The different groups were randomized into one of four treatments: air exposure (control), 3.0 ppm., 1.0 ppm., and 0.3 ppm. Exposures were made for periods of 4 hours daily, 5 days a week, except for holidays, until 90 exposures had been made over a period of 19 weeks. Food and water was allowed ad lib except during exposure. Food consisted of standard laboratory animal pellets, obtained from the Animal Supply Company an from Braun Knecht Heimann. The animals were handled daily during the five exposures per week, and inspected for unusual behavior, signs of toxicity, or intercurrent disease on each exposure. No specific treatment was rendered the animals when they were found to have respiratory tract irritation incidental to the exposure. The animals were weighed as a group at the beginning of the experiment, approximately every two weeks thereafter, and before sacrifice.

During the experiment, as animals died, they were autopsied, examined grossly, and sections of appropriate organs taken for histologic examination. At the conclusion of the experiment, surviving animals were sacrificed. Individual rats were sacrificed one at a time following anesthetization with ethyl ether and opening of the abdominal cavity by exsanguination through the abdominal aorta. Blood was

withdrawn under carefully applied negative pressure into a 10 ml. syringe. Determinations were made according to standard clinical laboratory procedures for the amount of hemoglobin, numbers of differential cells, total white cell count, and total blood urea. Methemoglobin values were not determined since no significant quantities of hemoglobin were found in any of the animals dying during the course of the exposure to the highest concentrations.

After exsanguination, the visceral and thoracic cavities were opened completely and gross inspection was made of all of the organs. The heart, lungs, liver, and kidneys of all female rats were weighed, and in the case of male rats, the testes as well. These tissues together with the thyroid, trachea, adrenal glands, pancreas, section of the stomach, bladder, and the genital organs were then prepared for microscopic examination by obtaining 2-3 mm. slices of appropriate area and placing them in formaldehyde. They were subsequently blocked, cut, stained, and examined by the consulting pathologist. Determination of the total cellular count of the bone marrow of the right femur was made, according to the method of Gerarde. Any tumors present were also dissected and appropriate sections taken.

Based on the data obtained above, determinations were then made of the mean weight gain, mortality, organ/body weight ratios of certain organs and of the incidence of the gross and microscopic pathological findings among the different groups.

3.4 SINGLE VAPOR EXPOSURES OF RATS TO SATURATED VAPORS

35 male, Long-Evans rats, weighing between 250 and 300 gms. were exposed for varying lengths of time to air saturated with vapors of HNE. Chemical determination indicated this level to average 13.2 ppm. during the course of the experiment. Subgroups of 5 rats each were removed commencing at 5 minutes after exposure and subsequently at 10, 20, 40, 80, and 160 minutes. Survival was noted, together with the time of death when it occurred. Surviving animals were sacrificed after 2 weeks, their lungs were examined for gross evidence of pathology and sections taken for microscopic examination.

3.5 REPEATED VAPOR EXPOSURES OF MICE

70 male Swiss-Webster mice obtained from the Animal Supply Company of Napa, California, were housed in the Laboratory for a period of two weeks. From this group 60 were chosen, discarding the 5 highest and 5 lowest in weight. The selected group were divided into four subgroups of 15 each, and housed in shoe-box cages, containing sawdust. They were allowed food and water ad lib, except during the time of exposure. Exposures were made in stainless steel exposure cages, the animals being separated in individual compartments. Exposure was made for a period of 4 hours to the pre-determined concentrations of 3.0, 1.0, and 0.3 ppm. The control group was air-exposed. The cages were checked daily for mortalities and dead mice were removed, autopsied, or, on weekends, frozen and later autopsied. At the conclusion of the experiment, surviving animals were weighed

and mean weights determined. They were then sacrificed by decapitation, following ether anesthesia, and the heart, liver, kidneys, and lungs were dissected free from adventitious tissue, blotted dry, and weighed. Organ/body weights were calculated from this data. Mortality data was summarized, and mean weight gains calculated. Since the previous experiment with rats had shown no significant changes in the blood picture, blood clinical studies were not carried out on this species. Following autopsy, the tissues were inspected grossly, abnormal appearing organs removed, and sections of the liver, kidneys and lungs taken for microscopic study.

3.6 REPEATED VAPOR EXPOSURES OF RABBITS

16 male albino rabbits, weighing between 2.2 and 3.0 kg. were obtained from a local animal supplier. These animals were housed for two weeks prior to induction into the experiment. As all appeared healthy, and gained weight normally, at the end of this time, they were randomized into 4 groups of 4 each, and housed singally one to a cage. They were fed a diet of standard laboratory rabbit pellets, augmented by greens twice weekly. Exposures were made to the same levels as those used with the other species. The animals were inspected daily for evidence of untoward effects. Their weight at the beginning and the conclusion of the 25 exposures was determined, at which time they were sacrificed. Autopsies were done on all animals as they died during exposure, and on sacrificed animals at the conclusion of the experiment. The animals were sacrificed by a blow on the cervical spine after which their juglar veins were cut. Organ-

body weights were determined on the lungs, of the surviving animals. Blood was obtained terminally for a clinical study and determination of the urea/nitrogen values.

3.7 REPEATED VAPOR EXPOSURES OF GUINEA PIGS

32 male guinea pigs were obtained from the Diablo Laboratories, and housed for a one-week period prior to induction into the experiment. As all animals appeared healthy at the time they were randomized into groups of 8 each, and these groups randomized among the 4 exposure treatments. Exposures were made daily with the animals being confined in the stainless steel compartments in the exposure chamber.

Exposures were terminated at the end of the 5th week, since there was only 1 surviving animal in the vapor exposed group. Since there were no significant survivals, blood work was not done on the guinea pigs, nor was it possible to obtain weight gain or organ/body weight ratios.

3.8 REPEATED SKIN APPLICATIONS TO RABBITS

Six albino rabbits, weighing between 2.0 and 3.0 kg. were delipitated with a hair clippers, and their skins allowed to heal from cuts and abrasions for a period of 72 hours. Following this, there were designated 5 spots on the rabbit's skin, randomly located over the back. On 2 of these, five drops of hexane were applied, and on the other 3 spots, five drops of a saturated solution of HNE in hexane. The material was inuncted in the skin and at the end of 1 hour, all areas were sponged with hexane. 20 applications were made in this manner

over a period of 5 weeks. Skin irritation scores were made by the standard method of Draize, readings were recorded for skin irritation at the end of a 1 hour period of application, prior to removing the excess material, and 22 hours later, prior to the next application. The degree of erythema and edema was rated on the Draize scale, which rates on an arbitrary scale from 0 to 8.

3,9 HUMAN SENSORY RESPONSES

Nine subjects, taken from a group of volunteers were exposed to varying concentrations of HNE vapors, and their sensory responses recorded initially on exposure and at minute intervals thereafter for a period of 5 minutes. The number of individuals undergoing an exposure at one time varied from 5 to 8. Exposures took place in the 15,000 liter exposure chamber into which were metered appropriate concentrations of HNE vapors. Analyses were made before entry to the chamber, during the residence time and for short periods thereafter. The subjects were unaware of the concentration to which they were being exposed. These were known only to the analyst. Nominal concentrations ranged from 0.25 to 2.0 ppm. The chamber was equipped with circulating fans, a communicating system, and a one-way view miror. The subjects were under constant observation, but did not know when they were being observed. The sensory responses of eye irritation, nose irritation, pulmonary discomfort, olefactory cognition, and central nervous system effects were recorded on the scale usually used in this laboratory. This varies over a five-fold range, including: "No recognition, Slight, Moderate, Severe, or Extreme" responses.

4.0 RESULTS

In paragraphs 4.1 through 4.3 there appears a narrative description summarizing the results obtained in the different experiments. The majority of data is presented in tabular form. References are made to these tables in each section.

4.1 MEASUREMENT OF DIFFERENT VAPOR CONCENTRATIONS

The mean vapor concentrations at the different exposure levels checked extremely well with the nominal concentrations. The 3.0 ppm nominal level averaged 3.17, the 1.0 ppm averaged 0.92, and the 0.3 ppm averaged 0.36. Due to alterations in ambient air temperature it was not possible to control the levels exactly; however, two standard deviations from the mean encompassed values which did not exceed the nominal concentration by 50%. Absolute variations for the two lower concentrations were actually very small.

4.2 MEASUREMENT OF DIFFERENT LOTS OF HNE

Batch L--II was taken as the standard for comparison with the two other batches which were analyzed. Batch L-VIII contained 105% HNE, as compared to the standard, where the lot having no batch number contained 107% HNE. No other batches were requested to be analyzed.

4.3 REPEATED VAPOR EXPOSURE OF RATS

The findings relative to the exposure of male and female rats are contained in paragraphs 4.30 - 4.39. For ease of comparison with the results obtained through testing other species, these data are described in

separately numbered paragraphs. The study on rats was of longer duration and survival ratios allowed more observations to be made than with other species.

4.31 BEHAVIOR

Inhalation of HNE vapors did not produce any characteristic alteration in behavior pattern of male or female rats. In both sexes prior to death, there was observed general decreased activity and failure to respond to stimuli, however, this was no different than the behavior generally noted in animals with any acute disease or intoxication state. The behavior was compatible with that seen in acute pulmonary edema and pneumonia, in that respiratory rates were generally increased and breathing appeared labored.

4.32 GROWTH

No significant depression in the growth rate of male or female rats was seen at either 0.3 or 1 ppm throughout the experiment. At the 3.0 ppm level, the weights of surviving animals at the end of the 6th week was significantly less than those of the controls, for both males and females. Following cessation of exposure of the one surviving female exposed to 3.0 ppm there was a rapid regain of body weight, and the terminal weight of this single animal was equivalent to that of the control. On autopsy of this animal, however, the lungs were found to be markedly enlarged, and microscopic examination showed peribronchiolitis and patchy bronchopneumonia. The majority of animals that died during the experiment showed a

weight loss in comparison with the control group at that time, and frequently with their own starting weight as well, Tables 1 and 1A.

4.33 MORTALITY

One of 15 animals in the female controls, and two of the 15 male controls died during the 90 day exposure period. There were no significant differences in mortality among the 0.3 and 1.0 ppm groups from the controls in either male or female groups. Fifteen of 16 females in 3.0 ppm had died by the 7th week of exposure, and all 14 males had died by the 6th week of exposure. There was no significant difference in total mortality of the male and female groups at 3.0 ppm, although the mortality rate was slightly accelerated in the case of the males. Mortality figures are summarized in Tables 3 and 4.

4.34 ORGAN/BODY WEIGHT

No significant differences in the organ/body weight ratios of the heart, liver or kidneys occurred among the three groups and the control. The lungs of animals exposed at the 1.0 ppm level were significantly heavier ($p < .05$) than those of the controls. The lungs of the single female rat surviving at 3.0 ppm were also heavier, but statistical comparisons could not be carried out due to the limitation in sample size. Lungs of female rats at the 0.3 ppm were heavier, but not significantly different from the control at the .05 level of significance (Table 8). In the case of the male rats, no significant difference in organ body ratios was seen in the heart, liver, kidney, and testes. In the 1.0 ppm group the lungs again were significantly heavier than those of the control group. There was an

excellent check in the mean values of all other organ/body weight ratios (Tables 8 and 9).

4.35 BLOOD COUNTS AND BONE MARROW

No significant differences were seen in the hemoglobin levels or in the differential counts of either male or female rats in the different exposure groups as compared with the control values. In the case of female rats, there was a significant increase in the total white blood cell count at both 1.0 and 0.3 ppm, and in the bone marrow counts at these levels as well. In the male rats this difference in white blood cell counts occurred at the same exposure levels, although the bone marrow count of only the 0.3 ppm group was significantly higher than those of the control. We interpret this data as indicating an increased activity of the anti-infective defenses of the rats. These were probably stimulated by the respiratory tract irritation with resulting tendency for pulmonary infection, since, as will be noted, spotty bronchopneumonia is quite frequent in these groups exposed to the higher levels of HNE concentrations. It will be noted that in the differential count, there is present in rats a preponderance of lymphocytes, and a relative decrease in neutrophils, a reversal of the ordinary distribution seen in man. Total white blood cell count in males and females (control group) reflects the levels generally seen in this strain of rat and are in keeping with values found in previous experiment in this laboratory. The relatively high levels of total nucleated bone marrow cells in the vapor exposed groups indicates no depression of the ability to produce red cells and granulocytes and no significant adverse

effects on the blood have been elicited by this treatment (Tables .2 and 13). It was not possible to demonstrate the presence of methemoglobin in the blood of the highest exposed group, and this physiologically altered pigment is apparently not formed due to exposure to HNE.

4.36 BLOOD UREA NITROGEN

Blood urea nitrogen values were elevated in both male and female rats at 1.0 ppm and 0.3 ppm. levels of HNE exposure in comparison with the controls. The mean values of male and female control rats were, for experimental purposes, identical. There was a moderate but significant elevation in the vapor exposed groups. This is probably a reflection of the altered liver and kidney changes noted on micorscopic examinations, and indicates a moderate disfunction of the kidneys, secondary to the exposure (Table 15).

4.37 GROSS PATHOLOGY

Duffuse enlargement of the lungs, termed as emphysema by us, was the gross pathological effect most commonly seen in male and female rats dying during the weeks of exposure. Pulmonary edema was frequently present also. In the latter cases, the cut surfaces frequently oozed a pink thin fluid. Congestion of the liver with darkening of the pigment was seen also with frequency. Other incidental findings are noted in Tables 16 and 17. Findings at autopsy of male rats at sacrifice at the termination of the experiment were generally not related to the lung. No growth deviations appeared in the control animals, and at 1.0 ppm only two animals had a generalized emphysema. The incidental finding in other male rats was

that of mesentery tumor, which occurred in two animals in the control group, five at 0.3 ppm, and five at 1.0 ppm. level. These were approximately 1 cm in diameter, oval, and generally located near the caecum or suspended in the mesentery. They were firm, non-encapsulated and cutting revealed a whitish to pink mass. Microscopic findings later identified them as lymphnodes with an acute inflammatory response, the cause of which was not determined. In female rats, nine of the animals exposed to 1.0 ppm had some gross pathology in the lung, predominantly emphysema, but bronchiectasis and sacculation were also noted. Only one control animal was observed to have enlargement of one of the lung lobes. At 0.3 ppm, no female rats had gross lung pathology; however, nine animals were noted to have the mesentery tumors, and one had a liver which was more friable than usual. A few other gross findings unrelated to the treatment were observed (Tables 21 and 22).

4.38 MICROSCOPIC PATHOLOGY

The major portion of microscopic pathology was confined principally to three tissues: the lungs, liver, and kidneys. Occasionally microscopic changes were also noted in the spleen, heart, and lymphnodes. As regards animals which died during the weeks of exposure, the most common pathology referable to the lungs in female rats was bronchopneumonia. On examination of these tissues 15 or 16 female rats and 4 of 14 male rats had this change. In two female rats interstitial pneumonia and congestion were also noted at the 3.0 ppm level. One or the other of these latter two findings was noted in 6 of the 7 male rats at the top level also. Seven of

the male rats which died also had a peribronchiolitis most commonly noted in the top level of exposure. A common accompaniment of the bronchopneumonia in the female rats was congestion of the liver and focal necrosis of the kidneys. Two rats, one male and one female in the top group, also developed an endocarditis. The mesenteric tumors described on gross pathology were found to be lymphnodes showing varying degrees of acute inflammatory response and increased lymphocyte production. Some nodes had hemorrhaged and derangement of the normal lymph structure was noted (Table 24).

All of the animals which were sacrificed at the end of the experiment were noted to have peribronchiolitis. This occurred in the control as well as the exposure groups. In addition, however, bronchopneumonia and interstitial pneumonia were noted with frequency in both the 0.3 and 1.0 ppm groups of female rats. This condition was not seen in any of the control animals, male or female. Bronchopneumonia also appeared in all of the male rats exposed to 0.3 and 1.0 ppm. Interstitial pneumonia was present in all male rats at the 1.0 ppm level. No significant liver or kidney changes were noted in female rats, however, all of the male rats exposed to 0.3 ppm had varying degrees of focal granuloma. This granuloma occurred in 4 of the 10 control animals as well, and is probably related to an incidental tapeworm or an endoparasite infection. It is not felt to reflect a toxic effect of the HNE. There were no other incidental findings of significance other than the presence of the mesenteric tumors which were described previously. Essentially free of pathology were the reproductive organs, gastrointestinal tract, thyroid, trachea, pancreas and bladder. (Table 25).

4.40 SINGLE VAPOR EXPOSURE OF RATS TO SATURATED VAPORS

All rats exposed for 5 or 10 minutes recovered, though the latter group showed slight dyspnea on removal from the chamber. At the 20 minute exposure, 3 of 5 animals survived. One died six hours after the start of the exposure, and the second 24 hours later. At the 40 minute exposure period, only one animal lived, death occurring at the same time period as in the 20 minute exposure.

At 80, 120, and 320 minutes no animals survived. Death occurred in the 90 minute group 2.5 hours after commencing the exposure, and all were dead at 3.5 hours following termination of the exposure. At the 160 and 320 minute exposures all animals died between the third and fourth hours of exposure.

On sacrifice of the remaining animals, 20 days later, there was no significant pulmonary pathology seen on gross autopsy. On microscopic examination there was a patchy bronchopneumonia, though no significant fibrosis in the surviving animals. No liver or kidney changes were apparent grossly or microscopically.

4.50 REPEATED VAPOR EXPOSURES OF MICE

Sub-paragraphs 4.51 though 4.56 summarize the observations noted in mice. Observations were made relative to behavior, growth, mortality, organ/body weight ratios, and gross and microscopic pathology.

4.51 BEHAVIOR

No alterations in general behavior were noted in mice exposed to various vapor concentrations in comparison with the control groups.

On exposure to pulmonary irritants, mice frequently show increased activity and excitement. This was noted only in the top exposure group, 3.0 ppm, in these experiments.

4.52 GROWTH

The mean weight gain of all groups was less than that of the controls, but only at 3.0 ppm was this significantly different. At that level there was an actual decrease in the mean weight of surviving animals in comparison with their initial starting weight. During the time of exposure the control grpup increased in weight 24% over their initial starting weights (Table 2).

4.53 MORTALITY

There were no significant differences in mortality between the control group and those exposed at 0.3 and 1.0 ppm; however, at 3.0 ppm there were only five survivors of the 15 original animals exposed, and the mortality was significantly higher in that group. Six of the mice at the 3.0 ppm level died at the fourth and fifth weeks. Mice were slightly more resistant than rats to exposures of the higher levels of HNE (Table 5).

4.54 ORGAN/BODY WEIGHT RATIOS

There were no significant changes in the organ/body weight ratios of the hearts, livers, and kidneys of the exposed mice. No differences in the lung weights were noted at 0.3 ppm level, but at both the 1.0 and 3.0 ppm levels there was a significant increase in lung weight. The weight at the 3.0 ppm level was nearly double the value of the control group. This

was due in part to the lower body weights of the surviving animals. This increase in lung weights we interpret as indicating an increased fluid retention and tissue hypertrophy in response to irritation.

4.55 GROSS PATHOLOGY

Not all of the animals which died at 3.0 ppm were suitable for autopsy, and observations were made on only 8 of the 10. Of those animals which died during the 1st week, no abnormalities of the lung were apparent grossly. In fact, only 3 of the 10 animals which died showed evidence of lung pathology in terms of congestion, emphysema, or hemorrhage In two animals gross pathology indicated by white cyst-like areas on the liver were noted. The findings in general were not significantly related to the exposures. At the time of sacrifice, there were minimal gross pathological changes noted. Emphysema was questionably present in one animal (Tables 19 and 23).

4.56 MICROSCOPIC PATHOLOGY

No significant microscopic pathological changes were seen in control mice or those exposed to 0.3 ppm and 1.0 ppm levels in animals which died during the exposure period; however, at the 0.3 ppm level 4 of 6 mice had congestion of the lungs, and 3 also showed focal necrosis of the liver and kidneys. In tissues obtained on sacrifice, the control mice were exceptionally free of any pathology. In only one animal was there an abnormal finding; in this case there was evidence of repair and regeneration of the liver. All of the animals exposed to 0.3 ppm showed peribronchiolitis, and similarly, there was 100 per cent involvement of the

lungs at 0.3 and 1.0 ppm levels with interstitial pneumonia. Broncho-pneumonia was not seen in this species. Also not noted in the mice were accompanying liver and kidney damage at the time of sacrifice (Tables 24 and 25). The rest of the tissues of the exposed mice showed no significant abnormality, and the inflammatory lymphnodes seen in the rat species were not identified in the mice.

4.60 REPEATED VAPOR EXPOSURES OF RABBITS

Observations on the rabbits included behavior, growth, mortality and limited blood work. The latter includes determination of blood urea nitrogen. It was not feasible to obtain quantitative bone marrow in these species, so this was not done.

4.61 BEHAVIOR

No unusual behavior patterns were noted in the rabbits with the exception of general uneasiness at even low vapor concentrations.

4.62 GROWTH

There were no surviving animals in the 1.0 and 3.0 ppm groups, and since weights were determined only at the beginning and termination of the experiment, there were no observations relative to these two groups At 0.3 ppm there was a slight decrease in weight gain which was not significantly different from the initial weight or from the changes in the control group.

4.63 MORTALITY

Two animals in the control group died during the fifth and sixth weeks of exposure, while only one rabbit died in the 0.3 ppm group.

No significant mortality differences will be seen in these groups, therefo
All animals exposed at the highest levels (3.0 ppm) died during the first week of exposure, and it was noted that they had extreme respiratory difficulty following single exposures. We concluded that the rabbits were more sensitive to the vapors of HNE than the rats and mice.

4.64 ORGAN/BODY WEIGHTS

The terminal mean lung/body weight ratio for the control animals was 0.45%, and that of the group exposed to 0.3 ppm, 0.68%. While the group exposed to HNE had a heavier lung weight, the number of animals surviving was too small to justify conclusions based upon the available data.

4.65 BLOOD COUNTS

The total white cell blood counts of the surviving rabbits in the 0.3 ppm group were double that of the control values. There was no essential difference in the hemoglobin concentrations, however. The differential counts also showed a slight increase in the numbers of eosinophils present in vapor-exposed groups in comparison with the controls. As in the case of rats, the predominant cell in the peripheral blood of these animals was the lymphocyte. No essential changes were seen in the cell distribution, and there did not seem to be any significant effect on the peripheral blood through the treatment offered. No animals survived in the two higher levels of exposure, and consequently no data is available for comparison in this group (Table 14).

4.66 BLOOD UREA NITROGEN

No significant differences occurred in the blood urea nitrogen levels between the control group and the three surviving animals which had received 0.3 ppm. (Table 15).

4.67 GROSS PATHOLOGY

Seven of the 8 animals exposed to the vapors of HNE showed gross evidence of pulmonary pathology. This included mottling of the lung, emphysema, marked pulmonary edema with hemorrhagic blood fluid, and lobar pneumonia. No other significant gross pathological findings were noted in exposed animals. Two control animals died. In one there was some evidence of liver pathology; both had shown evidence of diarrhea; and there was a bronchial pneumonia present in one animal. On sacrifice of the surviving rabbits, there was a finding of encysted parasites in the liver of the control animal, and a lung cyst with caseation in one exposed to 0.3 ppm. These findings were not felt to relate to the treatment, but indicated incidental endoparasitic infection (Tables 18 and 23).

4.68 MICROSCOPIC PATHOLOGY

All of the rabbits which died during the exposure to 3.0 or 0.1 ppm. showed extensive bronchopneumonia. In addition, there was congestion in 5 of these. At autopsy, the 0.3 ppm animals showed peribronchiolitis. This was not seen in the control animals. In general, the amount of changes in the lungs of rabbits was more extensive, involving a greater total of lung volume than was noted in the other species, and death at the higher levels was more rapid.

4.66 BLOOD UREA NITROGEN

No significant differences occurred in the blood urea nitrogen levels between the control group and the three surviving animals which had received 0.3 ppm. (Table 15).

4.67 GROSS PATHOLOGY

Seven of the 8 animals exposed to the vapors of HNE showed gross evidence of pulmonary pathology. This included mottling of the lung, emphysema, marked pulmonary edema with hemorrhagic blood fluid, and lobar pneumonia. No other significant gross pathological findings were noted in exposed animals. Two control animals died. In one there was some evidence of liver pathology; both had shown evidence of diarrhea; and there was a bronchial pneumonia present in one animal. On sacrifice of the surviving rabbits, there was a finding of encysted parasites in the liver of the control animal, and a lung cyst with caseation in one exposed to 0.3 ppm. These findings were not felt to relate to the treatment, but indicated incidental endoparasitic infection (Tables 18 and 23).

4.68 MICROSCOPIC PATHOLOGY

All of the rabbits which died during the exposure to 3.0 or 0.1 ppm. showed extensive bronchopneumonia. In addition, there was congestion in 5 of these. At autopsy, the 0.3 ppm animals showed peribronchiolitis. This was not seen in the control animals. In general, the amount of changes in the lungs of rabbits was more extensive, involving a greater total of lung volume than was noted in the other species, and death at the higher levels was more rapid.

4.7 REPEATED VAPOR EXPOSURE OF GUINEA PIGS

The experience on the exposure of the guinea pigs was not as satisfactory as had been hoped; and the heavy mortality in all groups including the control interferred with the accumulation of significant quantities of data. Ill animals were treated with antibiotics, without success. Because no animals were sacrificed, it was not possible to obtain satisfactory blood specimens for blood count, growth rates were not determinable and organ/body weight ratios could not be calculated.

4.71 BEHAVIOR

There was no unusual behavior in the case of the guinea pigs, except for increased nasal discharge and acute respiratory difficulty in pigs in the two upper groups, following even the first day's exposure. There was no evidence of central nervous system stimulation or depression aside from the accompanying depression with respiratory infection.

4.72 MORTALITY

By the end of the third week, 50% or more of each group exposed to the vapors of HNE had died. None of the control animals had succumbed by this time. The time for 100% mortality was the fourth week in the case of the 3.0 and 1.0 ppm animals, and the fifth week in the case of the 0.3 ppm animals. All the control animals had also died by the end of the sixth week. It has been our experience in the past, that when respiratory tract infections develop in guinea pigs, there will be almost a complete involvement of the entire colony housed within the same laboratory. It was our conclusion that the irritating vapors may have activated a

naturally occurring latent respiratory tract organism, which then caused generalized pulmonary infection.

All of the control group appeared to be essentially healthy until the latter part of the 4th week, when the respiratory tract infection which had killed the majority of the other animals became active in this group as well. The weight changes were not obtained on all animals, but when noted, in all cases there did appear a decrease from the original weight. In some this amounted to as much as 45%.

4.73 GROSS PATHOLOGY

In almost all cases, there was evidence of lung pathology. This consisted of emphysema, thoracic hemorrhage, bronchial and lobar pneu nonia, bronchiectasis, and pleural effusion. In a few instances there was noted enlargement of the heart, and in one animal exposed to 3.0 ppm there was what appeared to be a pericarditis.

4.74 MICROSCOPIC PATHOLOGY

Observations following microscopic observation confirmed the presence of diffuse bronchopneumonia, and pneumonitis in all animals. There were areas of marked alveolar distention, though no evidence of what would be equivalent to an emphysema in human pulmonary pathology. No significant lesions were seen in the livers, kidneys or other tissues.

4.80 REPEATED SKIN APPLICATION TO RABBITS

The individual irritation scores following application of HNE are summarized in Table 26 A and B. The minimum score achieved in all rabbits was zero, and the maximum was two. The maximum irritation

obtainable by this reading is 8, and the score of one would show very mild irritation. In four of the six rabbits there was a slightly greater irritation score in the case of the hexane plus HNE than appeared with the hexane control alone. In two of these there was no significant difference. The mean score for hexane was less than one in all but 4 of the 18 final mean readings. Three of these occurred on rabbit 6, and none reached a score of 1.2. It can be concluded from these observations, then, that continual exposure to HNE will produce little, if any, skin irritation, and that repeated contact should not present a significant skin irritation problem.

4.90 HUMAN SENSORY RESPONSES

The majority of the subjects who were tested at the 0.25 ppm concentration level showed no response at all, and in none of the categories was the response greater than "slight". At 0.5 ppm eye irritation of moderate intensity occurred in more than half of the subjects, and more than half also were able to recognize a slight but distinctive odor. No significant nasal irritation occurred at this level.

Eye irritation was noted by all subjects to a moderate or greater degree at 1.0 ppm, and one response of "extreme" was recorded. Nasal irritation was much less, with 5 of the 7 subjects indicating no more than slight nasal irritation at this level. The odor was recognized by the majority of the subjects as being moderately intense at 1.0 ppm and above. On exposure to the highest concentration, eye irritation was again the response with the highest scoring, all subjects indicating "moderate" to "severe" irritation. Olefactory cognition was not of a high level at this concentration; however, the nasal irritation was only slight.

The sensory modality most commonly affected on exposure to HNE vapors, therefore, was eye irritation, and this could be considered to serve as a significant warning at levels of 1.0 ppm and above.

No persons exposed showed any significant degree of pulmonary discomfort, and what has been related as such by five of the subjects who responded positively in this category at the four levels of exposure, was probably in reality moderate pharyngeal irritation only.

The odor of the compound was only fairly useful as an indication of its presence. While being recognized by some of the subjects at the lowest concentration tested, the majority did not recognize the presence of any material at this level. At the highest concentration, less than half of the volunteers responded, and then with only slight olefactory stimulation.

The central nervous system effects were only noted as "slight", and these consisted primarily of a slight headache, during the post-exposure period. The discrepancies noted,insofar as greater responses were sometimes obtained at lower levels, is consistent with the results generally seen in a panel where there exists varying degrees of sensitivity between the different subjects. If, during the experiment, the chance distribution of the more sensitive subject does not re-expose him at a higher concentration, his greater positive response is removed from the total summation of positive responses. In none of the subjects tested was there any significant retrogression of responses on continued exposure, so that a lesser degree of recognition occurred at a higher concentration.

Based on this data, it would appear that the best evidence of warning of the presence of undesirable concentrations of HNE would be eye

irritation. This, however, is not entirely satisfactory insofar as some subjects did not recognize this even at concentrations as high as 0.5 ppm.

5.0 DISCUSSION

As a result of the experiments carried out in this study, it may be concluded that HNE vapors are extremely toxic substances capable of giving rise to chronic intoxication on prolonged exposures. The principal toxicologic effect is the production of respiratory tract irritation with resulting pulmonary edema and chemical pneumonitis. In addition, in man it would be anticipated that chronic bronchitis, interstitial pneumonitis and bronchopneumonia would result. It is also quite possible that a bronchiolitis of an obstructive type, emphysema, and bronchiectasis might follow prolonged exposure to unsafe levels. The compound is capable under certain conditions of high exposure, of giving rise to a focal necrosis of the liver and the kidney. There was no significant kidney pathology at the 0.3 level of exposure as measured by frequency of structural alterations, however, the blood urea nitrogen levels were elevated in both male and female rats. The significance of this elevation in view of normal kidney structure at this level, is not clear; it does suggest that there may be some moderate effect on the physiological integrity of the kidney. There did not appear to be any essential differences between species in regard to their response to graded vapor concentrations; mice were slightly more resistant and guinea pigs least resistant of the four species. There is an early onset of respiratory tract inflammation leading to an acute and overwhelming pulmonary edema with a well-defined time relationship on exposure to saturated vapors.

Based on these experiments, it is apparent that environmental conditions that would result in such conditions must be avoided. Sensory

warning properties are present, and while distinctive, they are not of sufficient intensity to, in themselves, relate an unsafe concentration. Eye irritation is the most reliable evidence of the presence of the material. This is rather surprising insofar as there is a definite action on the lower respiratory tract as well. In general, compounds possessing acute eye irritating properties and which are therefore quite water soluble, act primarily on the upper respiratory tract in low concentrations.

5.1 MECHANISM OF ACTION

The mechanism of action of this substance is apparently one of causing surface irritation and disruption of the normal cellular integrity of the lining of the respiratory tract, increase in mucous secretion, and pulmonary edema in the capillary alveolar areas. In addition there is produced an extravasation of fluid into the alveolar sacs with the typical end result of pulmonary insufficiency. With the resulting high incidence of bronchopneumonia, it is quite likely that ciliary activity is also de-- creased secondarily, and that there is created a suitable background for bacterial invaders.

Somewhat to our surprise, it was not possible to demonstrate the presence of methemoglobin formation in any of the experiments, and we have concluded that there is, therefore, no interference with oxygen transport through the creation of this more stable form of hemoglobin. Liver and kidney damage is not commonly seen with agents which are primarily irritants of the respiratory tract, and we noted that there appeared changes almost entirely under circumstances which gave rise to overwhelming pulmonary insufficiency and death. While some nitro

compounds do cause direct cellular hepatic damage, they are not generally of the aliphatic type. We postulate that the action on both the hepatic cells and tubular cells of the kidney is one of direct intoxication, and is not secondary to oxygen lack. The compounds have no apparent effect on any other essential body functions, and the treatment rendered one overcome by the vapors would be similar to that for other irritant agents affecting the lower respiratory tract, such as oxides of nitrogen and phosgene.

5.2 POTENTIAL HAZARDS

Insofar as the toxicity hazard is concerned, we would conclude that there is present a moderate degree of hazard for a person exposed to the vapors of this material. As the environmental temperature increases the vapor pressure of this compound rises rapidly, and there is a proportionately greater quantity of the material in the ambient air. We do not see that the eventual hazard, therefore, would relate to the production of acute and subacute respiratory tract irritation, and methods suitable for protecting against this exposure should be followed and are suggested in Section 5.3. We do not see that there is any hazard from systemic toxicity relating to other major body systems, though liver and kidney function should be followed. There is also a remarkable freedom of skin irritating action, and we do not see the likelihood of either primary irritation or sensitization from the compound.

5.3 RECOMMENDED PROCEDURES FOR SAFE HANDLING

Based on our experience in industrial toxicology, we would recommend that a safe handling program be established to confine the vapors of this material so that the ambient concentration would not exceed 0.1 ppm in the air. At such a level there would certainly be no discomfort in terms of eye or respiratory tract irritation, and unsafe levels so far as respiratory tract damage would be avoided. Since saturated vapors at elevated temperatures would probably reach approximately 15 ppm, this is a recommended dilution factor of 150:1.

In persons who must work in close contact with large quantities of this material, it would appear desirable to have adequate ventilation to maintain concentrations not greater than those described above. In some instances, when for one reason or another adequate ventilation cannot be obtained, there must be local protection, either through the use of a canister mask equipped with an all purpose canister, or ventilation supplied through an air line, provided elevated concentrations are likely to be reached. No special type of protective clothing is needed, since neither percutaneous absorption nor skin irritation is a potential problem. The potential hazards relative to flammability and explosiveness are not considered as part of this report.

6.0 SUMMARY

(1) A chronic vapor toxicity study was carried out on four species of animals, extending for a period of 90 exposures in male and female rats, and 25 exposures in mice, guinea pigs and rabbits.

(2) Levels of exposure were 3.0, 1.0 and 0.3 ppm for all species.

(3) The top level of exposure, 3.0 ppm, was lethal to the majority of species. No significant effect on mortality occurred at the 1.0 ppm level for rats or mice, but was present in the case of guinea pigs and rabbits.

(4) Mean weight gain was decreased in mice at the 3.0 ppm level. There were insufficient survivors among other groups to make significant observation relative to the effect on weight.

(5) The chief toxic effect of HNE vapors in all species was production of pulmonary tract irritation and resulting pulmonary pathology.

(6) Organ/body weight ratios were used as an index of toxicity and showed significant changes in the male and female rats exposed to 1.0 ppm and in mice exposed to 3.0 and 1.0 ppm.

(7) Examination of the blood indicated no deleterious effect on red or white cell production, the distribution of different types of white cells, or the activity of the bone marrow in any of the species.

(8) Measurement of blood urea nitrogen indicated an increase in this value at both 1.0 and 0.3 ppm in the case of rats. The significance of this finding is equivocal in terms of kidney disfunction at the lower level of exposure, since the structural integrity of the kidney was intact.

(9) Gross pathologic changes in all species were most commonly seen in the lung and there was present at varying levels evidence of bronchiolitis, bronchopneumonia, interstitial pneumonia and congestion. In addition, some alterations were noted in the liver and kidneys of specific groups, generally those animals dying from the higher levels of

exposure. A number of other structural changes were observed. These were not felt to be due to the experiment and there was no general deleterious effect on other organ or tissue structures.

(10) Chronic skin irritation tests indicated the compound to be a relatively non-irritating substance, and no specific hazard relative to continuous skin contact was noted.

(11) Sensory response studies indicate that eye irritation is the most useful index of exposure to the vapors of HNE, with moderate to extreme irritation appearing at 1.0 ppm. Nasal irritation and olefactory recognition are relatively less useful criteria in estimating the presence of the material.

(12) Continued exposure to the vapors saturated with HNE is undesirable and may prove lethal in a period as short as 30 minutes.

(13) The mechanism of action of this compound is primarily that of a pulmonary tract irritant; there may be some influence on the liver and kidney as well.

(14) Safe handling procedure would necessitate establishment of a work situation which would allow exposures at no greater than 0.1 ppm. for prolonged periods of time, and concentrations on the order of 5-10 ppm should be permitted for only a few minutes.

Table 1. Mean Weights of Male and Female Rats During Chronic Exposures to Three Concentrations of HNE

Week of Exposure	Group							
	Control		0.3 PPM		1.0 PPM		3.0 PPM	
	no.	av.wt.(gm)	no.	av.wt.(gm)	no.	av.wt.(gm)	no.	av.wt.(gm)
Females								
(Initial)	15	172	15	171	15	167	16	155
3rd week	15	192	15	176	15	180	16	144
6th week	15	214	15	208	15	206	6	152
9th week	14	227	15	219	15	213	1	188 *
11th week	14	239	15	226	15	235	1	200
14th week	14	260	15	239	14	244	1	238
17th week	14	254	15	237	14	246	1	259
(Terminal)	14	261	14	255	14	243	1	260
Males								
(Initial)	12	244	15	217	15	220	14	212
3rd week	12	271	15	254	15	245	11	211
6th week	10	330	15	325	15	300	3	231
9th week	10	350	15	336	14	324	0	--
11th week	10	382	15	362	14	332		
14th week	10	406	15	384	14	356		
17th week	10	414	14	396	12	381		
(Terminal)	10	424	14	412	12	377		

* exposure stopped

Table 1-A. Mean Weight Gain for Male and Female Rats After Chronic Exposure to HNE

Sex	Exposure Group	Initial Weight (gm)	Terminal Weight (gm)	% Gain
Females	Control	172	261	51.74
	3.0 PPM	155	260	67.74[a]
	1.0 PPM	167	243	45.50
	0.3 PPM	171	255	49.12
Males	Control	244	424	73.77
	1.0 PPM	220	377	71.36
	0.3 PPM	217	412	89.86

[a] Only one survivor in this group; exposures were stopped after fifth week

Table 2. Initial and Terminal Mean Weights of Mice and Rabbits in Chronic Exposures to Three Concentrations of HNE

Date	Control		0.3 PPM		1.0 PPM		3.0 PPM	
	no.	av.wt.(gm)	no.	av.wt.(gm)	no.	av.wt.(gm)	no.	av.wt.(gm)
Mice								
8/21/61 (Initial)	15	21.3	15	21.8	15	23.1	15	22.7
10/2/61 (Terminal)	13	28.0	14	25.5	12	26.8	5	19.0*
Rabbits								
8/21/61 (Initial)	4	2272.5	4	2979	4	2415	4	2452
10/2/61 (Terminal)	2	2330	3	2873	0	—	0	—

*significantly different from controls

Table 3. Mortality of Female Rats During Chronic Exposure to Three Concentrations of HNE

Week of Exposure	Group							
	Control		0.3 PPM		1.0 PPM		3.0 PPM	
	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived
Before		15		15		15		16
First		15		15		15		16
Second		15		15		15		16
Third		15		15		15	1	15
Fourth		15		15		15	3	12
Fifth		15		15		15	4	8
Sixth		15		15		15	6	2
Seventh	1	14		15		15	1	1
Eighth		14		15		15	exposure discontinued	1
Ninth		14		15		15		1
Tenth		14		15		15		1
Eleventh		14		15		15		1
Twelfth		14		15		15		1
Thirteenth		14		15	1	14		1
Fourteenth		14		15		14		1
Fifteenth		14		15		14		1
Sixteenth		14	1	14		14		1
Seventeenth		14		14		14		1
Eighteenth		14		14		14		1
Nineteenth		14		14		14		1

Table 3. Mortality of Female Rats During Chronic Exposure to Three Concentrations of HNE

Week of Exposure	Group							
	Control		0.3 PPM		1.0 PPM		3.0 PPM	
	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived
Before		15		15		15		16
First		15		15		15		16
Second		15		15		15		16
Third		15		15		15	1	15
Fourth		15		15		15	3	12
Fifth		15		15		15	4	8
Sixth		15		15		15	6	2
Seventh	1	14		15		15	1	1
Eighth		14		15		15	exposure discontinued	1
Ninth		14		15		15		1
Tenth		14		15		15		1
Eleventh		14		15		15		1
Twelfth		14		15		15		1
Thirteenth		14		15	1	14		1
Fourteenth		14		15		14		1
Fifteenth		14		15		14		1
Sixteenth		14	1	14		14		1
Seventeenth		14		14		14		1
Eighteenth		14		14		14		1
Nineteenth		14		14		14		1

Table 5. Mortality of Mice During Chronic Exposure to Three Concentrations of HNE

Week of Exposure	Group							
	Control		0.3 PPM		1.0 PPM		3.0 PPM	
	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived
Before		15		15		15		15
First	1 (accident)	14		15		15	3	12
Second		14		15		15		12
Third		14		15	1	14	1	11
Fourth		14	1	14	1	13	2	9
Fifth	1	13		14		13	4	5
Sixth		13		14	1	12		5

Table 6. Mortality of Rabbits During Chronic Exposure to Three Concentrations of HNE

Week of Exposure	Group							
	Control		0.3 PPM		1.0 PPM		3.0 PPM	
	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived
Before		4		4		4		4
First		4		4	1	3	4	0
Second		4		4	2	1		0
Third		4	1	3		1		0
Fourth		4		3	1	0		0
Fifth	1	3		3		0		0
Sixth	1	2		3		0		0

Table 7. Mortality of Guinea Pigs During Chronic Exposure to Three Concentrations of HNE

Week of Exposure	Group							
	Control		0.3 PPM		1.0 PPM		3.0 PPM	
	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived	Died During Week	Survived
Before		8		8		8		8
First		8		8		8	3	5
Second		8	1	7	1	7		5
Third		8	3	4	5	2	3	2
Fourth	4	4	3	1	2	0	2	0
Fifth	1	3	1	0		0		0
Sixth	3	0		0		0		0

Table 8. Summary of Individual Organ-Body Weight Ratios of Different Organs for Female Rats Exposed to Different Concentrations of HNE

Identification		Organ/Body Weight (%)			
Exposure	Rat No.	Heart	Liver	Kidneys	Lung
Control	1	0.31	3.62	0.70	0.43
	2	0.26	3.25	0.63	0.52
	3	0.30	3.33	0.76	0.55
	4	0.30	3.02	0.72	0.47
	5	0.30	3.14	0.64	0.54
	6	0.30	2.92	0.63	0.55
	7	0.29	3.28	0.60	0.42
	8	0.29	3.30	0.71	0.46
	9	0.29	2.96	0.74	0.53
	10	0.28	3.34	0.72	0.49
	11	0.27	3.40	0.54	0.49
	12	0.27	3.47	0.67	0.49
	13	0.25	2.60	0.57	0.39
	14	0.24	3.33	0.61	0.48
	Mean	0.28	3.21	0.66	0.486
3 PPM	1	0.29	3.08	0.68	0.65
1 PPM	1	0.32	3.60	0.73	0.73
	2	0.35	2.68	0.74	0.90
	3	0.26	2.85	0.60	0.70
	4	0.30	2.91	0.63	0.74
	5	0.29	3.48	0.69	0.98
	6	0.27	3.37	0.72	0.59
	7	0.31	3.18	0.70	0.69
	8	0.28	2.72	0.66	0.59
	9	0.32	3.30	0.62	0.87
	10	0.28	2.77	0.58	0.73
	11	0.34	3.20	0.73	0.64
	12	0.29	3.10	0.68	1.24
	13	0.32	3.57	0.66	0.61
	14	0.32	2.69	0.66	0.72
	Mean	0.30	3.10	0.67	0.766*
0.3 PPM	1	0.28	3.26	0.73	0.71
	2	0.28	3.64	0.63	0.68
	3	0.33	3.09	0.72	0.66
	4	0.28	3.09	0.63	0.44
	5	0.25	2.88	0.54	0.51
	6	0.28	3.24	0.60	0.47
	7	0.28	3.24	0.60	0.47
	8	0.36	3.53	0.71	0.74
	9	0.32	3.47	0.75	0.61
	10	0.28	3.46	0.72	0.52
	11	0.30	2.95	0.56	0.51
	12	0.26	3.24	0.60	0.48
	13	0.26	3.48	0.64	0.54
	14	0.31	3.08	0.72	0.46
	Mean	0.29	3.24	0.66	0.558

*significantly different from controls

Table 9. Summary of Individual Organ-Body Weight Ratios of Different Organs for Male Rats Exposed to Different Concentrations of HNE

Identification		Organ/Body Weight (%)				
Exposure	Rat No.	Heart	Liver	Kidneys	Lung	Testes
Control	1	0.26	2.88	0.68	0.45	0.85
	2	0.24	4.14	0.70	0.40	0.82
	3	0.27	3.22	0.67	0.51	0.93
	4	0.31	3.32	0.69	0.52	1.04
	5	0.24	3.05	0.65	0.40	0.77
	6	0.27	3.10	0.72	0.44	0.81
	7	0.28	2.67	0.64	0.42	0.76
	8	0.23	3.49	0.63	0.44	0.72
	9	0.28	3.02	0.81	0.41	0.95
	10	0.32	2.66	0.65	0.37	0.78
	Mean	0.27	3.155	0.68	0.44	0.84
1 PPM	1	0.28	2.91	0.72	0.79	0.97
	2	0.28	3.09	0.75	0.87	0.51 (1 testis)
	3	0.27	3.41	0.61	0.74	0.92
	4	0.28	2.89	0.66	0.98	0.78
	5	0.29	3.12	0.66	0.57	0.85
	6	0.34	2.81	0.62	0.78	0.96
	7	0.31	2.72	0.71	0.78	1.03
	8	0.27	2.96	0.65	0.70	0.87
	9	0.26	2.70	0.72	0.56	0.83
	10	0.24	3.10	0.66	0.76	0.81
	11	0.29	2.98	0.71	0.80	0.84
	12	0.25	2.85	0.67	0.58	0.88
	Mean	0.28	2.96	0.68	0.74*	0.89
0.3 PPM	1	0.28	3.50	0.74	0.42	1.00
	2	0.28	3.29	0.65	0.53	0.96
	3	0.27	2.93	0.60	0.43	0.77
	4	0.30	3.53	0.75	0.40	0.88
	5	0.26	3.34	0.63	0.44	1.02
	6	0.28	3.13	0.62	0.35	0.95
	7	0.30	3.10	0.71	0.40	0.87
	8	0.28	3.25	0.70	0.43	0.83
	9	0.25	2.78	0.66	0.36	0.90
	10	0.27	2.93	0.69	0.35	0.78
	11	0.25	2.89	0.63	0.37	1.04
	12	0.25	3.00	0.63	0.37	0.76
	13	0.25	2.99	0.66	0.32	0.73
	14	0.27	3.28	0.71	0.37	0.76
	Mean	0.27	3.14	0.67	0.396	0.81

*significantly different from controls

Table 10. Summary of Individual Organ-Body Weight Ratios of Different Organs for Male Mice Exposed to Different Concentrations of HNE

Identification		Organ/Body Weight (%)			
Exposure	Rat No.	Heart	Liver	Kidneys	Lung
Control	1	0.52	6.00	1.38	0.76
	2	0.41	6.75	1.56	0.50
	3	0.47	5.27	1.17	0.57
	4	0.39	5.86	1.54	0.64
	5	0.38	5.97	1.24	0.69
	6	0.34	6.09	1.56	0.75
	7	0.45	5.64	1.64	0.77
	8	0.47	6.34	1.47	0.69
	9	0.42	6.18	1.52	0.61
	10	0.34	5.54	1.20	0.57
	11	0.48	7.85	1.63	0.81
	12	0.50	6.73	1.55	0.64
	13	0.48	8.24	1.48	0.86
	Mean	0.43	6.34	1.46	0.68
3 PPM	1	0.48	4.57	1.48	1.13
	2	0.54	6.31	1.85	1.46
	3	0.50	5.05	1.65	1.00
	4	0.45	5.60	1.55	1.10
	5	0.47	4.32	1.63	1.68
	Mean	0.488	5.17	1.63	1.274*
1 PPM	1	0.37	4.60	1.40	0.93
	2	0.53	5.77	1.70	1.07
	3	0.39	4.64	1.36	0.71
	4	0.45	5.32	1.36	0.95
	5	0.45	5.40	1.45	1.05
	6	0.41	5.07	1.45	0.83
	7	0.39	4.68	1.29	0.84
	8	0.50	4.82	1.46	0.82
	9	0.43	5.96	1.39	1.00
	10	0.53	5.37	1.47	0.90
	11	0.42	5.58	1.35	0.85
	12	0.44	5.28	1.40	1.28
	Mean	0.44	5.21	1.42	0.936*
0.3 PPM	1	0.42	5.17	1.42	0.83
	2	0.43	5.68	1.36	0.64
	3	0.56	5.88	1.44	0.64
	4	0.42	5.83	1.33	0.75
	5	0.45	5.50	1.45	0.70
	6	0.34	5.38	1.31	0.56
	7	0.40	5.73	1.33	0.57
	8	0.39	5.31	1.35	0.54
	9	0.37	5.70	1.40	0.63
	10	0.43	5.07	1.32	0.64
	11	0.43	6.14	1.48	0.67
	12	0.43	4.93	1.50	0.57
	13	0.47	5.68	1.47	0.68
	Mean	0.43	5.54	1.40	0.648

*significantly different from controls

Table 11. Summary of Individual Lung-Body Weight Ratios of Control Rabbits and Those Exposed to 0.3 PPM of HMX

Exposure	Rabbit No.	Terminal Body Wt (gm)	Lung Wt (gm)	Ratio Lung/Body (%)
Control	1	2325	12.76	0.55
	2	2335	8.08	0.35
	Mean	2330	10.42	0.45
0.3 PPM	1	2850	18.91	0.66
	2	2835	12.97	0.46
	3	2935	27.55	0.94
	Mean	2873	19.81	0.68

Table 12. Summary of the Individual Blood Counts on a Series of Ten Female Rats Taken From Each Exposure Group

Identification		Determination						
Exposure	Rat No.	Bone Marrow cells/femur	W.B.C.	Hemoglobin gm %	Mono-cytes	Lympho-cytes	Neutro-phils	Eosino-phils
Control	1	109500	4400	13.4	2	69	23	6
	2	98200	4350	12.8	2	71	25	2
	3	58600	2600	12.3	1	63	36	0
	4	71200	2650	12.8	7	75	16	2
	5	66700	3150	12.9	1	83	16	0
	6	111500	4450	12.8	0	83	17	0
	7	97300	4650	12.1	3	54	42	1
	8	108000	5600	11.7	2	51	44	3
	9	32200	3200	12.8	0	79	15	6
	10	97500	5800	11.9	2	48	50	0
	Mean	85070	4085	12.55	2.0	67.6	28.2	2.0
3 PPM	1	99000	7700	15.4	1	63	35	1
1 PPM	1	129000	3150	12.8	1	82	15	2
	2	137000	5400	15.6	6	78	10	6
	3	102000	8000	13.1	4	88	6	2
	4	135000	8100	12.9	1	74	24	1
	5	131000	5400	12.9	0	84	15	1
	6	124000	7000	12.6	5	76	17	2
	7	98000	5750	13.5	4	49	45	2
	8	124000	5300	12.7	3	84	9	4
	9		6500	12.0	3	57	36	4
	10	112000	5350	12.8	6	81	11	2
	Mean	121333*	6000*	13.1	3.3	75.3	18.6	2.6
0.3 PPM	1	104000	9000	12.1	1	63	36	0
	2	129000	6600	12.1	4	71	23	2
	3	156000	7850	11.7	9	87	3	1
	4	116000	9350	12.1	1	71	24	4
	5	104000	5650	12.5	1	80	18	1
	6	143000	7050	12.9	4	48	46	2
	7	124000	8000	11.2	0	91	9	0
	8	221000	6950	12.6	5	60	32	3
	9	162000	8500	12.4				
	10	135000	7900	13.4	3	63	34	0
	Mean	139400*	7683*	12.3	3.1	70.4	24.7	1.4

*significantly different from controls

Table 13. Summary of the Individual Blood Counts on a Series of Ten Male Rats Taken From Each Exposure Group

Identification		Determination						
Exposure	Rat No.	Bone Marrow cells/femur	W.B.C.	Hemoglobin gm %	Mono-cytes	Lympho-cytes	Neutro-phils	Eosino-phils
Control	1	132000	7700	12.9	1	49	49	1
	2	141000		13.3	2	74	18	6
	3	139300	6150	13.9	2	67	24	7
	4	90000	4000	13.1	3	65	31	1
	5	109500	7400	12.5	1	69	29	1
	6	234000	7150	12.0	1	47	52	0
	7	86200	3600	13.2	0	68	30	2
	8	128200	5750	13.1	1	69	30	0
	9	126000	7300	13.0	0	48	51	1
	10	204000	7200	13.0	2	63	34	1
	Mean	139020	6250	13.0	1.3	61.9	34.6	2.0
1 PPM	1	114000	10750	10.9	9	48	42	1
	2	109000	8550	15.0	12	73	15	0
	3	131000	9450	13.1	4	63	33	0
	4	149000	7300	13.2	10	75	14	1
	5	132000	7300	14.2	3	81	14	2
	6	154000	7600	14.5	1	84	10	5
	7	140000	6350	14.0	6	77	16	1
	8	82000	7150	13.6	5	71	24	0
	9	100000	6250	14.7	6	64	28	2
	10	98000	7000	13.1	7	62	30	1
	Mean	120900	7770*	13.6	6.3	69.8	22.5	1.3
0.3 PPM	1	208000	14100	12.4	2	70	28	0
	2	223000	12850	13.6	4	69	21	6
	3	202000	12700	13.6	2	72	24	2
	4	180000	12230	12.3	2	41	55	2
	5	151000	11850	12.0	1	70	28	1
	6	145000	8400	12.8	4	59	36	1
	7	157000	9150	13.6	1	68	27	4
	8	255000	10050	12.8	0	68	30	2
	9	117000	14000	12.3	2	51	47	0
	10	161000	10000	13.2	3	71	23	3
	Mean	179900*	11533*	12.9	2.1	63.9	31.7	2.1

*significantly different from controls

Table 14. Summary of the Individual Blood Counts on Control Rabbits and Those Exposed to 0.3 PPM of HME

Identification		Determination						
Exposure	Rabbit No.	W.B.C.	Hemoglobin gm %	Mono-cytes	Lympho-cytes	Neutro-phils	Eosino-phils	Baso-phils
Control	1	1550	11.0	3	76	8	13	0
	2	7000	12.9	2	76	1	21	0
	Mean	4275	11.95	2.5	76	4.5	17	0
0.3 PPM	1	9800	13.9	4	73	0	22	1
	2	5650	13.2	6	79	0	15	0
	3	19050	10.6	4	42	0	54	0
	Mean	11500	12.6	4.7	64.7	0	30.3	0.3

Table 15. Summary of Individual Blood Urea Nitrogen Determinations in Female and Male Rats and Male Rabbits Exposed to Different Concentrations of HNE

Identification		Blood Urea Nitrogen gm/100 ml		
Exposure	Animal No.	Rats Female	Rats Male	Rabbits
Control	1	25	26	32
	2	25	20	<10
	3	25	26	
	4	25	21	
	5	23	23	
	6	<10	25	
	7	33	18	
	8	26	30	
	9	10	20	
	10	31	23	
	Mean	23.3	23.2	
3 PPM		28		
1 PPM	1	31	23	
	2	31	19	
	3	39	31	
	4	18	19	
	5	20	31	
	6	30	44	
	7	30	30	
	8	44	33	
	9	44	30	
	10	28	39	
	Mean	31.5*	29.7*	
0.3 PPM	1	31	25	<10
	2	23	30	25
	3	38	28	<10
	4	34	30	
	5	25	28	
	6	26	26	
	7	28	20	
	8	26	30	
	9	36	36	
	10	30	28	
	Mean	29.7*	28.1*	

*significantly different from controls

Table

Week Death
1st
1st
2nd
3rd
3rd
4th
4th
5th
5th
6th
7th
7th

a. Mea

b. Exp

c. Exp

Table 16. Gross Pathological Findings in Male Rats Which Died During Chronic Exposure to 3.0 PPM and 1.0 PPm of HNE

Week of Death	% Wt. Change [a]	Gross Findings [b]
1st	-47.6	Upper l. lobe caseous; lungs dilated and emphysematous; cut sections ooze
1st	-33.0	Lungs slightly emphysematous; cut sections ooze
2nd	-37.7	Lungs as above; liver mahogany colored, congested
3rd	-23.1	Lungs emphysematous; acute pulmonary edema
3rd	- 6.6	Lungs markedly enlarged, gorged with fluid, some apparent pneumonia; acute passive congestion of liver
4th	+13.2	Lungs emphysematous; heart dilated; liver dark
4th	+22.6	Markedly dilated lungs; liver dark; red blood elsewhere
5th	- 5.7	Lungs white, swollen, turgid; liver dark
5th	- 5.2	Nothing noted
6th	+13.2	Lungs greatly enlarged; liver dark
7th	-15.1	Lungs pink with red spots; liver dark
7th	-21.8	Lungs pink with darker spots [c]

a. $\frac{\text{Terminal Weight}}{\text{Mean Initial Weight}} \times 100$

b. Exposed to 3.0 PPM unless otherwise indicated

c. Exposed to 1.0 PPM

Table 17. Gross Pathological Findings in Female Rats Which Died During Chronic Exposure to 3.0, 1.0 and 0.3 PPM of HNE

Week of Death	% Wt. Change[a]	Gross Findings[b]
3rd	-34.2	Obstruction of descending colon; tumor; lungs slightly edematous
4th	-22.6	Lungs markedly emphysematous; viscera dark
4th	- 4.5	Lungs emphysematous; liver dark; marked rigor
4th	+ 9.7	Lungs markedly enlarged and oozing; liver dark; rigor
5th	+30.3	Lungs enlarged with dark red and white spots; liver dark
5th	+ 0.6	Lungs as above; fluid in thoracic cavity; liver dark
5th	+40.6	Lungs as above; liver dark
5th	+11.0	Nothing noted
6th	- 9.7	Lungs emphysematous
6th	- 6.5	Lungs as above; liver dark
6th	-23.2	Lungs white with some red mottling
6th	+33.5	Lungs pink with red mottling
6th	- 1.3	Nothing noted
7th	-27.1	Lungs pink with dark red spots; stomach, intestine empty
13th	+25.7	Lungs mod. enlarged--3 areas of red blotches on pleural surface; cut sections ooze mucous[c]
17th	-19.3	Lungs emphysematous[d]

a. $\frac{\text{Terminal Weight}}{\text{Mean Initial Weight}} \times 100$

b. Exposed to 3.0 PPM unless otherwise indicated

c. Exposed to 1.0 PPM

d. Exposed to 0.3 PPM

Table 18. Gross Pathological Findings in Rabbits Which Died During Chronic Exposure to Three Concentrations of HNE and Air Control

Week of Death	% Wt. Change[a]	PPM	Gross Findings
1st		3.0	Lungs mottled and appear emphysematous
1st	- 9.9	3.0	Markedly enlarged lungs with fluid; cut surfaces ooze bloody fluid
1st	-30.7	3.0	Lungs enlarged; cut surfaces ooze
1st	- 3.3	3.0	Lungs appear normal
2nd	-18.8	1.0	Patchy pneumonia; moderate amount of fluid
2nd	-10.1	1.0	Lower lobes consolidated
2nd	-31.1	1.0	Medial lobe hepatized, hemorrhagic; some edema, areas of emphysema, pneumonia
4th		1.0	Areas of pneumonia
3rd	-15.2	0.3	Lungs emphysematous, left lobes hemorrhagic; liver dark—acute passive congestion; heart dilated
5th		air	Severe diarrhea, emaciation; liver bile-stained; hard white spots (2mm); area of hepatization on lungs, severe congestion; edema fluid upon cutting
6th		air	Diarrhea; hemorrhagic patches on left lobe of lung

[a] $\frac{\text{Terminal Weight}}{\text{Mean Initial Weight}} \times 100$

Table 19. Gross Pathological Findings in Mice Which Died During Chronic Exposure to Three Concentrations of [illegible]

Week of Death	% Wt. Change[a]	PPM	Gross Findings
1st		3.0	Lungs appear normal
1st		3.0	" " "
1st		3.0	" " "
3rd		3.0	Lungs small, but appear normal
4th	-33.9	3.0	White spots on liver (parasites?); left kidney small with 1x2mm external growth; lungs normal
5th	- 7.5	3.0	Lungs somewhat congested
5th	-11.9	3.0	Lungs enlarged, with small blebs; hemorrhagic, emphysematous
5th	-25.1	3.0	Calcified liver with fibrin tags; lungs enlarged with pale areas; nodular growth on kidney; stripe with difficulty
3rd		1.0	Lungs enlarged, extended, hemorrhagic
4th	-12.8	0.3	White spots on liver (parasite?), spot on kidney; lungs appear normal

[a] $\frac{\text{Terminal Weight}}{\text{Mean Initial Weight}} \times 100$

Table 20. Gross Pathological Findings in Guinea Pigs Which Died During Chronic Exposure to Three Concentrations of HME and Air Control

Week of Death	% Wt. Change	PPM	Gross Findings
1st	- 7.6	3	Lungs slightly emphysematous
1st	-19.7	3	Nothing noted
1st	-11.6	3	Lungs moderately dilated
3rd	- 7.6	3	Lungs emphysematous, hemorrhages in thoracic cavity; pericardial sac full of fluid, enlarged heart; pneumonia in right lower and medial lobes
3rd		3	Lungs somewhat enlarged with fibrinous tags; some edema
4th	-43.7	3	Right lobe emphysematous, discolored, pneumonic; liver bile-stained
5th	-31.7	3	Hemorrhagic areas in lungs, bronchiectasis, heart, spleen enlarged
3rd		1	Lungs enlarged; hemorrhagic exudate in thoracic cavity
3rd		1	Extensive hemorrhage in thoracic cavity; lungs enlarged
3rd	-27.9	1	L. lower lobe emphysematous; kidney enlarged, mottled
3rd		1	Hemorrhage in thoracic cavity, areas of emphysema, congestion
4th		1	Thoracic cavity full of blood, some edema, with white spots
4th		1	Fluid in thoracic cavity; right lobes hyperemic, consolidated, left lobes emphysematous, consolidated
2nd	-44.9	0.3	Lungs emphysematous, congested, possibly pneumonic
3rd		0.3	Fluid in thoracic cavity, one lobe autolyzed, also liver
3rd		0.3	Blood in thoracic cavity, lungs enlarged
4th		0.3	Blood in thoracic cavity
4th	-37.8	0.3	L. lower lobe mod. pneumonic, slight bile-staining of liver
4th	-37.8	0.3	Left lobes moderately pneumonic
5th	-15.4	0.3	Lungs enlarged
4th	-27.2	Air	Patchy pneumonia upper l. lobe; gall bladder distended
4th	-40.9	Air	Pneumonic process l. lower lobe?; gall bladder distended
5th	-25.2	Air	Nothing noted

Table 21. Gross Pathology of Male Rats at Time of Sacrifice

Group	Rat No.	Gross Findings
Controls	6	Mesenteric tumor (1 cm diameter)
	10	Tumor in mesentery near caecum (1 x 3/4 cm)
0.3 PPM	1	Tumor in caecum (1 cm diameter)
	4	Five cysts (1½ cm diameter) in omentum
	5	Tumor (1 cm diameter)
	8	Two tumors (1 x 3/4 cm)
	11	Two small tumors in caecum
	12	Lungs small; small mesenteric tumor
	13	Lungs small
1.0 PPM	1	Right lower lobe of lung enlarged; mesenteric tumor (1x1 cm)
	2	Lungs emphysematous
	4	Mesenteric tumor (1x1½ cm)
	5	Lungs small, collapsed; mesenteric tumor (2x1 cm)
	7	Generalized emphysema
	8	Mesenteric tumor (1 cm diameter)
	9	Lungs small
	11	Mesenteric tumor (2 x 3/4 cm)

Table 2a. Gross Pathology of Female Rats at Time of Sacrifice

Group	Rat No.	Gross Findings
Controls	6	Lungs emphysematous in several lobes
	7	Tumor (1½ cm diameter) omentum small intestine
	8	" " " " " "
	10	Two caecal tumors (2 cm diameter), loculated; one portion hemorrhagic
	11	Tumor (1x2 cm) in omentum, loculated
0.3 PPM	1	Tumor (1x1½ cm) in mesentery near caecum, loculated
	2	Tumor (1½x1½ cm) " " " " "
	3	Tumor (1 cm diameter) " " " "
	4	Tumor (1½x2 cm) " " " " "
	5	Small tumor or cyst (½cm diameter) as above
	7	Tumor, thick-walled, solid
	9	Liver friable
	10	Three tumors in caecal area (2½x1½ cm)
	11	Small tumor (½x½ cm)
	12	Tumor (1½x½ cm)
	13	Tumor (3x2x1½ cm)
1.0 PPM	1	Possible emphysematous areas in right lower lobe
	2	Emphysematous area of 1/8" on lower lobe; lobe enlarged
	3	Some enlargement of several lobes
	4	Tumor in caecum (1x1 cm)
	5	Emphysematous lobes; upper left lobe especially marked
	7	Doubtful sacculation in left lower lobe of lung
	8	Stomach mucosa hemorrhagic; 2mm white scar on lesser curvature
	9	Emphysematous areas in lungs
	10	Left lower lobe emphysematous
	12	Frank bronchiectasis upper right lobe; hypertrophied
	13	Caecal tumor (1 cm diameter)
	14	Bronchiectasis of lower right lobe
3.0 PPM	1	Numerous tumors; lungs enlarged, emphysematous

Table 23. Gross Pathology of Mice and Rabbits at Time of Sacrifice

Group	Mouse No.	Gross Findings
Controls	11	Blood in abdominal cavity
	13	Cyst on liver; liver friable
0.3 PPM	8	Liver covered with punctate cysts; spleen enlarged; lungs pale
1.0 PPM	8	Possible bleb on lungs
3.0 PPM	1	Questionable emphysema
	2	Liver friable
	Rabbit No.	
Controls	1	Encysted parasites in liver
0.3 PPM	3	Large cyst (3x1¼ cm) on left lower lobe of lung; caseation of entire lung; several patches of calcification; cysts in liver

Table 24. Summary of Microscopic Pathologic Findings in Animals Which Died During Chronic Exposures to Three Concentrations of HNE

Identification			Number of Animals Showing Organ Pathology								
			Lungs				Liver			Kidneys	
No. Animals Examined		PPM	BP	PB	IP	C	FN	C	CLN	FN	Other
Female Rats	1	1.0	1						1		
	15	3.0	14		1	2		11		14	
Male Rats	1	0.3	1	1							
	6	1.0		1		2	2			1	
	7	3.0	3	5	2	4	5	4		4	2[I]
Mice	1	Control				1					
	1	0.3				1	1				
	1	1.0	1								
	6	3.0	1			4	3			3	
Rabbits	2	Control	1								1[IH]
	1	0.3					1				
	4	1.0	4			2		1			
	4	3.0	4			3	4			3	

BP=Bronchopneumonia; PB=Peribronchiolitis; IP=Interstitial Pneumonia; C=Congestion; FN=Focal Necrosis; CLN=Central Lobular Necrosis; I=Infarct; IH=Interstitial Hemorrhage

Table 25. Summary of Microscopic Pathologic Findings in Animals Which Survived Chronic Exposures to Three Concentrations of HNE

Identification			Number of Animals Showing Organ Pathology						
No. Animals Examined		PPM	Lungs			Liver		Kidneys	Omental Tumors
			BP	PB	IP	FG	FN	FN	
Female Rats	14	Control		14					5
	14	0.3	14	14	14				10
	14	1.0		14	14				2
	1	3.0	1	1					1
Male Rats	10	Control		10		4			2
	14	0.3	14	14		14			6
	12	1.0	12		12				5
Mice	13	Control							
	14	0.3		14			1		
	12	1.0			12				
	5	3.0			5				
Rabbits	2	Control							
	3	0.3		3					

BP=Bronchopneumonia; PB=Peribronchiolitis; IP=Interstitial Pneumonia; FG=Focal Granuloma; FN=Focal Necrosis

Table 26-A Individual Irritation Scores Following Application of HNE to Rabbits

Application	Time (hrs)	Rabbit 1					Rabbit 2					Rabbit 3				
		Hexane Control		Hexane + HNE			Hexane Control		Hexane + HNE			Hexane Control		Hexane + HNE		
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	2	1	2	2	2	0	0	±	±	±
2	1															
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	1	1	1	0	0	0	0	0	±	0	0	0	0
4	1	0	0	±	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	1	2	2	0	0	0	0	+	0	0	0	0	0
5	1	0	0	0	1	1	0	0	0	1	1	0	0	±	1	±
	24	0	0	±	1	±	0	0	1	0	1	0	0	±	±	±
6	1	±	±	1	1	1	0	0	1	1	1	0	0	1	1	1
	24	1	0	1	1	1	0	0	1	±	±	±	0	0	0	0
7	1	0	0	1	1	1	0	0	0	0	1	0	0	±	1	±
	72	0	0	*	*	*	*	*	*	*	*	0	0	*	*	0
8	1	0	0	±	±	±	±	0	1	1	1	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	+	1	0	0	0	0	0
9	1	0	0	±	±	±	0	0	±	±	1	1	1	1	1	1
	24	1	0	1	1	1	0	0	0	±	±	1	0	1	1	±
10	1	0	0	1	1	1	0	0	0	0	0	±	0	1	1	1
	24	1	0	1	1	1	0	0	0	0	±	1	0	1	1	±
11	1	±	±	1	1	1	0	0	0	0	0	1	0	1	1	1
	24	0	1	1	1	1	0	0	0	0	1	1	0	1	1	1
12	1	0	0	1	1	1	0	0	0	0	0	1	0	1	1	1
	72	0	1	1	1	1	0	0	0	0	0	1	0	1	1	1
13	1	0	0	1	1	1	0	0	1	0	0	0	0	1	1	1
	48	0	0	±	±	±	1	0	1	0	0	±	±	±	±	±
14	1	0	0	1	1	1	1	0	1	1	1	0	0	1	1	1
	24	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
15	1	0	±	1	1	1	1	0	1	1	1	0	1	1	2	1
	24	0	±	1	1	1	1	0	1	1	1	1	1	1	2	1
16	1	±	0	1	1	1	±	0	0	0	0	0	0	1	2	1
	72	±	±	1	1	1	±	±	±	±	±	1	0	1	1	1
17	1	1	1	2	2	2	±	±	±	±	±	1	1	1	2	1
	24	1	0	1	1	1	0	0	0	0	0	1	1	1	2	1
18	1	1	0	1	1	1	±	0	0	0	0	0	0	1	1	1
	24	±	±	1	1	1	±	±	±	±	±	1	1	1	1	1
19	1	0	±	1	1	1	0	0	0	0	±	1	0	1	1	1
	24	1	0	1	1	1	0	0	0	0	0	1	±	±	1	±
20	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1
	24	0	1	1	1	1	0	0	0	0	0	1	1	1	1	1
Minimum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Maximum		1	1	2	2	2	1	1	2	2	2	1	1	1	2	1
Mean		0.29	.23	.79	.84	.82	.26	.05	.37	.31	.47	.47	.24	.68	.84	.66

*impossible to read because of scaling

Table 26-B. Individual Irritation Scores Following Application of HNE to Rabbits

Application	Time (hrs)	Rabbit 4					Rabbit 5					Rabbit 6				
		Hexane Control		Hexane + HNE			Hexane Control		Hexane + HNE			Hexane Control		Hexane + HNE		
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	1	0	0	1	1	1	1	1	1	1	2	2	2	2	2
2	1															
	72	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
3	1	0	0	0	0	0	0	0	0	2	0	0	0	0	+	0
	24	0	0	0	0	0	1	0	±	1	±	1	0	1	½	1
4	1	0	0	0	0	0	+	0	+	1	0	+	0	+	+	+
	24	0	0	0	0	0	1	1	0	1	0	1	1	1	1	1
5	1	0	0	0	0	±	1	1	1	1	1	1	1	1	2	1
	24	0	0	0	0	±	1	±	1	1	1	2	1	1	1	1
6	1	0	0	+	+	+	1	1	2	2	2	1	2	1	2	2
	24	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
7	1	0	0	0	0	±	1	0	1	1	2	0	0	0	0	0
	72	*	*	*	*	*	*	*	*	*	*	*	0	*	*	*
8	1	0	0	1	±	±	0	0	0	0	0	0	0	1	2	2
	24	1	1	1	1	1	0	0	±	±	±	1	1	1	1	1
9	1	0	0	+	+	1	1	0	1	1	0	0	0	1	1	1
	24	1	0	1	1	1	2	1	0	2	0	0	0	1	1	1
10	1	1	1	1	2	1	1	0	1	1	1	0	+	2	2	2
	24	2	2	1	2	2	2	1	1	2	1	2	1	1	2	2
11	1	1	1	1	1	1	2	1	1	2	1	1	0	1	1	1
	24	2	1	1	1	1	2	1	1	2	1	1	1	1	1	1
12	1	2	1	1	1	1	1	1	1	1	1	0	0	1	1	1
	72	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1
13	1	0	1	1	1	1	1	1	+	1	1	0	0	1	1	1
	48	1	1	1	1	1	1	0	0	1	0	0	0	1	1	1
14	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1
	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	2	1	1	1	1	2	1
	24	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
	72	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
17	1	1	1	1	1	1	1	0	1	1	1	1	2	2	2	2
	24	1	1	1	±	1	0	0	0	0	0	1	1	1	1	1
18	1	1	±	1	0	1	0	0	1	1	1	1	1	1	1	1
	24	1	1	1	1	1	±	±	1	±	±	1	1	2	2	2
19	1	1	0	1	0	1	±	0	1	±	1	1	1	2	0	2
	24	1	1	1	0	±	1	0	1	0	1	1	1	1	1	1
20	1	1	1	1	1	1	±	±	1	±	1	±	1	1	1	1
	24	2	1	1	1	1	±	1	0	±	1	1	1	1	1	1
Minimum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Maximum		2	2	1	2	2	2	1	2	2	2	2	2	2	2	2
Mean		.74	.58	.68	.63	.76	.87	.45	.74	1.0	.74	.71	.71	1.03	1.16	1.11

*impossible to read because of scaling

Table 27. Summary of the Sensory Response Data to HNE

Exposure Concentration PPM	No. Subjects Exposed	Eye Irritation					Nose Irritation					Pulmonary Discomfort					Olfactory Cognition					CNS Effects				
		A	S	M	Se	E	A	S	M	Se	E	A	S	M	Se	E	A	S	M	Se	E	A	S	M	Se	E
0.25	8	6	2				6	2				6	2				6	2				5	3			
0.50	6	2		4			3	2	1			6					1	4	1			5	1			
1.0	7			4	2	1	2	3	2			5	2					2	4	1		6	1			
2.0	5			2	3		2	3				4	1					3	1	1		5				

A = absent

S = slight

M = moderate

Se = severe

E = extreme